Essentials of MEDICAL MICROBIOLOGY

Students Feedback
- Revolutionary book; crystal clear concepts and crisp presentation.
- Proud to have this book; far better than any others; boon for examination.
- Very well written book. A must for UGs, PGs and PGMEE aspirants.
- Very nice book. I wish I would have known about this book earlier.
- Gem of a microbiology textbook. Up-to-date info., short and precise.
- Absolutely brilliant, thanks authors, for this wonderful creation.
- All Microbiology MCQs of PGMEE exam (2015-2018) sessions—came from this book.

First Indian Clinical Microbiology Book
- Newer sections included-Hospital infection control and Clinical infective syndromes.
- Updates made in recent advances in laboratory diagnosis, treatment, prophylaxis and epidemiology.
- Newer topics included such as advances in sterilization (plasma sterilization, sterilization indicators), automations (MALDITOF, VITEK, BacT/ALERT), molecular methods (real-time PCR, LAMP and BioFire FilmArray) serological methods (IgG avidity test, ELFA, CLIA methods), newer emerging diseases (including Nipah virus and Zika virus).
- Bulleted format in concise, simple and lucid language.

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Essentials of Medical Microbiology

SECOND EDITION

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The Health Sciences Publisher
New Delhi | London | Panama
It is a proud moment for us. The excitement reaches its crazy heights as our sleepless nights of last six months have come to an end and the second edition of Essentials of Medical Microbiology is released.

The first edition went viral among students and faculty; was highly appreciated. Many have described it as ‘block buster’, ‘revolutionary book’, ‘game changer book’, ‘boon for exam’, catering to the need of all: UG, PGME aspirant and PGs, etc. The second edition has been designed in a similar fashion taking all your feedback and suggestions into consideration. The newer concepts and recent advances incorporated are as follows:

- **Section 1: General Microbiology section** has been updated with addition of topics such as recent Nobel laureates, latest changes in nomenclature, newer sterilization methods/topics (plasma sterilization, ETO, etc., with an update on sterilization indicators, a special note on CSSD), newer automations (MALDIMFH, VITEK, BacT/ALERT VIRTUO, newer molecular methods (LAMP, Real-time PCR, Biofire FilmArray, etc.), newer antimicrobial agents such as fifth generation cephalosporins, fosfomycin and colistin, latest antimicrobial susceptibility testing (AST) methods including automated AST methods.

- **Section 2: Immunology section** has been updated with newer serological methods such as ELFA technology (Vidas system), use of chemiluminescence immunoassay in diagnostic microbiology, IgG avidity, ELISA, etc. All the schematic figures are redrawn to make the concept crystal clear.

- **Section 3: In Systematic Bacteriology section**, many topics are thoroughly updated such as pneumococcal vaccine, MRSA, VRSA, VRE, anthrax vaccine, diphtheria vaccine, epidemiology of meningococcal meningitis, *Clostridioides difficile* infection, elimination of neonatal tetanus, tuberculosis (thorough updation on newer investigations such as GeneXpert, MGIT, TrueNat, Drug Susceptibility Testing methods, latest RNTCP guideline on diagnosis and treatment of TB), leprosy (including a note on newer leprosy vaccine introduced by Government of India), melioidosis, scrub typhus, yaws (including yaws eradication program), leptospirosis, etc. The epidemiology, laboratory diagnosis, and treatment have been thoroughly updated in all the chapters. Many newer photographs have been incorporated for better understanding.

- **Section 4: Virology section** needs a special mention. We must say that this section underwent a major update. As we all know that virology is the emerging area of microbiology with lots of changes keep happening every year. We updated all the chapters with latest epidemiology, laboratory diagnosis and treatment data from standard references with incorporation of many new photographs, tables, and flow charts. The topics which underwent major update include influenza (epidemiology, laboratory diagnosis with special emphasis on interpretation of real-time PCR, vaccine), measles and rubella (epidemiology and laboratory diagnosis), arboviruses (new topics such as Zika virus, SFTS virus; update of existing topics such as chikungunya, dengue, JE virus, yellow fever, etc.), hepatitis (update in epidemiology, laboratory diagnosis, treatment of HBV, and HCV), HIV (update in epidemiology, NACO strategy, progressors, treatment and PEP), Nipah virus (including the Kerala outbreak, 2018), poliomyelitis (update in vaccine schedule, epidemiology and end game strategy and GPEI), rabies (epidemiology, laboratory diagnosis and prophylaxis) and many more.

- **Section 5: Mycology section** has been updated with recent advances/changes in epidemiology, nomenclature of fungi, laboratory diagnosis, and treatment of various fungal diseases. Many new photographs have been incorporated.

- **Section 6: Hospital Infection Control** has been added as a new section in this edition. It was a long-time demand from the readers from all the parts of our country. Infection control practices are very poorly followed in India and lack of awareness at undergraduate level is the main reason behind it. As first of its kind, we have introduced this section which addresses several areas of infection control such as hospital-acquired infection surveillance, biomedical waste (according to 2016 guideline, with 2018 amendment), needle stick injury, antimicrobial stewardship and environmental surveillance (it is a revised chapter of the first edition chapter, ‘Bacteriology of Water, Air, Surface, Milk and Food’). We propose the faculty to keep at least 6-7 dedicated theory hours and two hours of practical classes for this section. Basic knowledge on infection control practice is the minimum thing expected from any MBBS doctor.
Section 7: Clinical Microbiology (Infective Syndromes) chapter of first edition has been expanded to a new section with each syndrome has been divided into a separate chapter. Each chapter begins with clinical case scenarios. This section will bridge the gap between bench microbiology and clinical microbiology. This is a major requirement in today's date. The subject 'Microbiology' in India is moving towards 'Clinical Microbiology and Infectious Disease.' Having a clinical-oriented section is of great help, which will provide the students and readers a bird's eye view on clinical aspects of various infectious diseases.

Annexures: Quality control in microbiology has been added as a new annexure. All the previous annexures have been thoroughly updated.

MCQs: More number of MCQs have been incorporated in every chapter as requested by most readers.

Inclusion of more tables, flowcharts, real images and schematic diagrams—for better understanding.

Most features of the first edition have been maintained: such as concept of more content-less pages, concise, bulleted format and to-the-point text, simple and lucid language, separate boxes for summary of laboratory diagnosis and treatment for quick review, clinical case-based essay questions at the end of each chapter.

As you know, human errors are inevitable; and no book is immune from it. We would request all the readers to provide any errata found and also valuable suggestions and updates via e-mail.

We are confident and hoping that you all will fall in love with this revised second edition of the book.

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## Section 1  General Microbiology

1. Introduction and Bacterial Taxonomy  
   page 3
2. Morphology and Physiology of Bacteria  
   page 9
3. Sterilization and Disinfection  
   page 31
4. Culture Media and Culture Methods  
   page 46
5. Identification of Bacteria  
   (Conventional methods, Automations and Molecular methods)  
   page 56
6. Bacterial Genetics  
   page 71
7. Antimicrobial Agents, Antimicrobial Resistance and Antimicrobial Susceptibility Testing  
   page 84
8. Microbial Pathogenicity  
   page 94

## Section 2  Immunology

9. Immunity (Innate and Acquired)  
   page 103
10. Antigen  
    page 111
11. Antibody  
    page 116
12. Antigen–Antibody Reaction  
    page 127
13. Complement  
    page 144
14. Structure of Immune System  
    page 151
15. Immune Responses: Cell-mediated and Antibody-mediated  
    page 168
16. Hypersensitivity  
    page 177
17. Autoimmunity  
    page 188
18. Immunodeficiency Disorders  
    page 193
19. Transplant and Cancer Immunology  
    page 199
20. Immunoprophylaxis and Immunohematology  
    page 208

## Section 3  Systematic Bacteriology

### Gram-positive cocci

21. *Staphylococcus*  
   page 217
22. *Streptococcus, Enterococcus* and *Pneumococcus*  
    page 228

### Gram-negative cocci

23. *Neisseria* and *Moraxella*  
    page 245
Gram-positive bacilli
24. Corynebacterium 253
25. Bacillus 260
26. Anaerobes (Clostridium and Non-sporing Anaerobes) 266
27. Mycobacteria
   (M. tuberculosis, Non-tuberculous mycobacteria and M. leprae) 281
28. Miscellaneous Gram-positive Bacilli
   (Actinomycetes, Listeria, Erysipelothrix and Tropheryma) 304

Gram-negative bacilli
29. Enterobacteriaceae-I
   (Escherichia, Shigella, Klebsiella, Proteus, Yersinia and others) 310
30. Enterobacteriaceae II: Salmonella 330
31. Vibrio and Aeromonas 341
32. Pseudomonas and Other Non-fermenters 352
33. Haemophilus and HACEK Group 359
34. Bordetella 366
35. Brucella 370
36. Miscellaneous Gram-negative Bacilli
   (Campylobacter, Helicobacter, Legionella, Pasteurella, Francisella and agent of Donovanosis, Rat-bite fever, Bacterial vaginosis) 376

Other group of bacteria
37. Spirochetes
   (Treponema, Borrelia and Leptospira) 386
38. Rickettsiae, Coxiella and Bartonella 403
39. Chlamydiae 413
40. Mycoplasma and Ureaplasma 421

Section 4  Virology
41. General Properties of Viruses 427

DNA viruses
42. Herpesviruses
   (Herpes simplex viruses, Varicella-zoster virus, Cytomegalovirus, Epstein-Barr virus and others) 449
43. Other DNA Viruses
   (Parvoviridae, Papillomaviridae, Polyomaviridae, Poxviridae, Adenoviridae and Bacteriophages) 463

RNA viruses
44. Myxoviruses and Rubella Virus
   [Orthomyxovirus (Influenza), Paramyxovirus (Parainfluenza, Mumps, Measles, Respiratory syncytial virus, Nipah virus and others)] 474
45. Picornaviruses
   (Poliovirus, Coxsackievirus and others) 494
46. Arboviruses
   (Chikungunya, Japanese encephalitis, Dengue, Yellow fever, Zika virus, Kyasanur forest disease and others) 502
47. Rhabdoviruses
   (Rabies Virus) 517
48. HIV and Other Retroviruses 524
49. Miscellaneous RNA Viruses
   [Rodent-borne (Hantaviruses, Arenaviruses), Filoviruses (Ebola virus, Marburg virus),
   Coronaviruses, Slow viruses, Rotavirus and other agents of viral gastroenteritis and Bornavirus] 539

Other group of viruses
50. Hepatitis Viruses 549
51. Oncogenic Viruses 566

**Section 5** Mycology
52. Medical Mycology 573

**Section 6** Hospital Infection Control
53. Hospital-acquired Infections 605
54. Major Hospital-acquired Infection Types: Surveillance and Prevention 613
55. Biomedical Waste Management 617
56. Needle Stick Injury Prevention and Management 621
57. Antimicrobial Stewardship 625
58. Environmental Surveillance (Bacteriology of Water, Air, Surface, Milk, and Food) 628

**Section 7** Clinical Microbiology (Infective Syndromes)
59. Normal Microbial Flora of Human Body 637
60. Urinary Tract Infections 641
61. Diarrheal Diseases 645
62. Meningitis 651
63. Blood Stream Infections 655
64. Respiratory Tract Infection 662
65. Miscellaneous Infectious Syndromes 667

**Annexures**
1. Annexure 1: Emerging and Re-emerging Infections 675
2. Annexure 2: Bioterrorism—Biological Warfare 677
3. Annexure 3: Laboratory-acquired Infections 679
4. Annexure 4: Zoonosis 680
5. Annexure 5: Quality Control in Microbiology 681

Index 685
Section Outline

21. Staphylococcus 217
22. Streptococcus, Enterococcus and Pneumococcus 228
23. Neisseria and Moraxella 245
24. Corynebacterium 253
25. Bacillus 260
26. Anaerobes (Clostridium and Non-sporing Anaerobes) 266
27. Mycobacteria 281
28. Miscellaneous Gram-positive Bacilli 304
29. Enterobacteriaceae-I 310
30. Enterobacteriaceae II: Salmonella 330
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32. Pseudomonas and Other Non-fermenters 352
33. Haemophilus and HACEK Group 359
34. Bordetella 366
35. Brucella 370
36. Miscellaneous Gram-negative Bacilli 376
37. Spirochetes 386
38. Rickettsiae, Coxiella and Bartonella 403
39. Chlamydiae 413
40. Mycoplasma and Ureaplasma 421
INTRODUCTION
Gram-positive cocci are classified into two families—Micrococcaceae and Streptococcaceae, differentiated by the catalase test. Micrococcaceae are catalase positive, gram-positive cocci arranged in tetrads or clusters; whereas Streptococcaceae are catalase negative gram-positive cocci, arranged in pairs or chains.

Family Micrococcaceae comprises of four genera—Micrococcus, Stomatococcus, Planococcus and Staphylococcus.
- Micrococcus species are skin commensals, usually not associated with human infections. They are 1–1.8 µm size, arranged in tetrads. As they are obligate aerobes, they show oxidative pattern in Hugh and Leifson’s oxidative-fermentative (OF) test
- Planococcus and Stomatococcus are not pathogenic to man
- Staphylococcus species are arranged in clusters, show fermentative pattern in oxidative fermentative test.
  - Among Staphylococcus species, S. aureus is the most pathogenic; it produces an enzyme coagulase which forms the basis of coagulase test
  - Whereas, other species do not produce coagulase and are called as coagulase-negative staphylococci (CoNS). They are rarely pathogenic to man; may cause infections in immunocompromised patients
  - S. epidermidis is the most common CoNS infecting man, followed by S. saprophyticus, S. lugdunensis, S. schleiferi, S. haemolyticus and S. warneri.

History
Staphylococcus was first observed in pus by von Recklinghausen (1871) and was first cultured in liquid medium by Louis Pasteur (1880).

It was named as Staphylococcus (in Greek, Staphyle means ‘bunch of grapes’ and kokkos means berry) by Sir Alexander Ogston (1880). Rosenbach (1884) named two species of staphylococci based on pigmentation of colonies as S. aureus (golden yellow colonies) and S. albus (white colonies). Later Passet (1885) named a third species as S. citreus (lemon yellow colonies).

STAPHYLOCOCCUS AUREUS

Staphylococcus aureus is catalase positive, coagulase positive, facultative anaerobe, non-motile, non-sporing and occasionally capsulated.
- They are spherical cocci, about 1 µm in diameter, arranged in grape-like clusters. This arrangement is due to cell-division in S. aureus; which occurs in multiple planes with daughter cells remain attached together
- It produces golden yellow pigmentation on nutrient agar and β hemolytic colonies on blood agar
- S. aureus is the most virulent species among staphylococci; produces infections which range from localized pyogenic infections to life-threatening systemic infections in man
- Its importance as human pathogen is greatly enhanced especially in hospital environment because of its ability to develop drug resistance.

Virulence Factors
S. aureus possesses an array of virulence factors as listed in Table 21.1.

Cell Wall Associated Factors
Like most gram-positive bacteria, the cell wall of Staphylococcus consists of a thick peptidoglycan layer and teichoic acid. S. aureus has additional factors in the cell wall, such as protein A and clumping factor.
Section 3  Systematic Bacteriology

### Peptidoglycan
Similar to other gram-positive bacteria, the peptidoglycan layer of *Staphylococcus* is thicker (15–80 nm, up to 100 layers thick).
- It confers rigidity to the cell wall and maintains the shape.
- It induces inflammatory response and also has endotoxin-like activity.

### Teichoic Acid
It is made up of ribitol phosphate polymers, helps in adhesion of cocci to mucosal surfaces and inhibits opsonization.

### Cell Surface Adhesins
- Clumping factor/bound coagulase—it is a fibrinogen binding adhesin; responsible for slide coagulase reaction.
- Fibronectin binding adhesin
- Collagen-binding adhesin.

### Protein A (SpA)
It is a 42 kDa polypeptide, encoded by *spa* gene. It is present in 90–99% of human *S. aureus* strains (especially the Cowan I strains).
- Protein A has many biological properties, such as anti-complementary, chemotactic, mitogenic, inhibition of opsonization and induction of platelet damage.
- **Mediates coagglutination reaction:** Protein A binds to Fc region of any IgG antibody, leaving Fab region free which binds to the corresponding antigen present in clinical samples (Detail described in Chapter 12).

### Microcapsule
Some strains of *S. aureus* have polysaccharide microcapsule, which inhibits phagocytosis by neutrophils. The capsular polysaccharides are zwitterionic, i.e. they have both negative and positive charges, which is a feature that is critical for abscess formation.

### Toxins

#### Membrane Active Toxins

**Hemolysins**
* *S. aureus* produces four distinct hemolysins—α, β, γ and δ hemolysins. They are membrane damaging toxins, act on red blood cells (RBCs) leading to hemolysis. They differ from each other by their action on RBCs of different animals, their lethal, dermonecrotic and leukocidal activity (Table 21.2).

**γ-hemolysin**
- It has three protein fragments which act together along with leukocidin to exhibit hemolytic activity.
- It is also lethal, leukocidal and dermonecrotic.

**β-hemolysin**
- It is sphingomyelinase in nature:
  - Lyses sheep RBC, but not human or rabbit RBC; this explains why hemolysis of *S. aureus* is better in sheep blood agar than human blood agar.
  - Exhibits hot-cold phenomenon, i.e. hemolysis starts at 37°C but becomes evident only after chilling.

**δ-hemolysin**
- It is detergent like (surfactant) action.
- Mediates coagglutination reaction: Protein A binds to Fc region of any IgG antibody, leaving Fab region free which binds to the corresponding antigen present in clinical samples (Detail described in Chapter 12).

**Leukocidins/Panton Valentine Toxin**
It is also called as Panton Valentine (PV) toxin; named after its discoverers.
- It has two components F (fast) and S (slow) based on their migration on carboxymethyl cellulose column.
- Both the fragments act synergistically with γ-hemolysin to damage leukocytes, RBCs and macrophages.
- **Synergohymenotropic toxins:** γ-hemolysin and PV toxin are called as synergohymenotropic toxins. Because they are not active individually, but in combination, they are capable of producing hemolytic and leukocidal activity. There are six combinations possible by the interaction between three fragments of γ-hemolysin with the two fragments of PV toxin.

### Table 21.3: Virulence factors of *Staphylococcus aureus*

<table>
<thead>
<tr>
<th>Cell wall associated factors</th>
<th>Toxins</th>
<th>Extracellular enzymes</th>
</tr>
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<tbody>
<tr>
<td>Peptidoglycan</td>
<td>Membrane active toxins</td>
<td>Coagulase</td>
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<tr>
<td>Teichoic acid</td>
<td>• Hemolysins—α, β, γ, δ</td>
<td>Heat stable thermonuclease</td>
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<td>Cell surface adhesins, e.g.</td>
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<td>Deoxyribonuclease</td>
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<tr>
<td>clumping factor</td>
<td>Epidermolytic toxin (exfoliative toxin)</td>
<td>Staphylokinase (fibrinolysin)</td>
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<tr>
<td>Protein A</td>
<td>Enterotoxins</td>
<td>Others—hyaluronidase, lipase, and protease</td>
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### Table 21.2: Hemolysins of *Staphylococcus aureus* and their activity

<table>
<thead>
<tr>
<th>Hemolysins</th>
<th>Activities</th>
</tr>
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<tbody>
<tr>
<td>α-hemolysin</td>
<td>It is inactivated at 70°C but again reactivated paradoxically at 100°C (This is because at 60°C α-hemolysin combines with a heat labile inactivator which gets denatured at 100°C) It possesses lethal, leukocidal, dermonecrotic, cytotoxic and neurotoxic activities</td>
</tr>
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</table>
| β-hemolysin| It is sphingomyelinase in nature:
  - Lyses sheep RBC, but not human or rabbit RBC; this explains why hemolysis of *S. aureus* is better in sheep blood agar than human blood agar
  - Exhibits hot-cold phenomenon, i.e. hemolysis starts at 37°C but becomes evident only after chilling |
| γ-hemolysin| It has three protein fragments which act together along with leukocidin to exhibit hemolytic activity |
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**Table 21.1: Virulence factors of *Staphylococcus aureus***

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<td>Others—hyaluronidase, lipase, and protease</td>
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</table>
CHAPTER 21  Staphylococcus

- PV toxin is expressed on MRSA (methicillin-resistant *Staphylococcus aureus*) strains, which are associated with the community acquired infections.

**Epidermolytic/Exfoliative Toxin (ET)**

This toxin is responsible for staphylococcal scalded skin syndrome (SSSS).

- **It comprises of two proteins:** ET-A (chromosomal, heat stable) and ET-B (plasmid coded, heat labile)
- **Staphylococcal scalded skin syndrome (SSSS)** often occurs in newborns and children, more often than adults
- Illness may vary from localized tender blisters and bullae formation to exfoliation and separation of outer epidermal layer leaving denuded underlying skin (the later is called as Nikolsky’s sign)
- The mucous membranes are usually spared
- Severe form in a newborn is called as Ritter’s syndrome; characterized by fever, lethargy, and irritability with poor feeding
- Milder forms—pemphigus neonatorum and bullous impetigo
- Epidermolytic toxin producing strains belong to *S. aureus* bacteriophage group II.

**Enterotoxin**

Enterotoxin is expressed by nearly 50% of *S. aureus* strains and is responsible for staphylococcal food poisoning.

- It is a preformed toxin (secreted in food before consumption) so that it can act rapidly. As a result, the incubation period is short (1–6 hours)
- **Site of action:** The toxin stimulates the vagus nerve and the vomiting center of the brain. It also appears to stimulate the intestinal peristaltic activity
- **Symptoms:** Staphylococcal food poisoning is characterized by nausea, vomiting, occasionally diarrhea, hypotension, and dehydration; however, there is no fever. Symptoms generally resolve within 8–10 hours
- Most common source of infection is a food handler, who is a carrier of *S. aureus*. There is no secondary spread
- Most common food items involved are milk products, bakery food, custards, potato salad, or processed meat
- It is a heat stable toxin and is resistant to gastric juice
- **Serotyping:** Enterotoxins can be typed into many serotypes (A–E, G–I, R–T and V)
  - Type A is most common to cause food poisoning
  - Serotype-F does not cause food poisoning; but causes toxic shock syndrome
  - Serotype-I, Q and U are enterotoxin—like toxins.
- *S. aureus* enterotoxins are also responsible for some cases of pseudomembranous colitis following use of broad spectrum antibiotics
- **Detection of enterotoxin** in food is carried out by ELISA or latex agglutination test or by detecting enterotoxin gene by multiplex PCR (polymerase chain reaction)
- Treatment is entirely supportive by correcting fluid and electrolyte imbalance.

**Toxic Shock Syndrome Toxin (TSST)**

This toxin is responsible for toxic shock syndrome (TSS). It has two subtypes—TSST-1 and TSST-2.

- TSST-1 is actually a staphylococcal enterotoxin. Enterotoxin F or pyrogenic exotoxin C is the most common type of TSST-1; rarely enterotoxin-B or C may also be associated
- TSST producing strains belong to *S. aureus* bacteriophage group I

**Risk factors:** Initially, toxic shock syndrome was reported from women using highly absorbent vaginal tampons during menstruation. Subsequently, TSS has been reported from both men and non-menstruating women as a complication of staphylococcal abscesses, osteomyelitis and post-surgical, traumatic or burn wound infections

**Pathogenesis:** TSST-1 gets absorbed into circulation from the tampons; then being a superantigen it stimulates the T-cells non-specifically (by binding to Vβ region of T-cell receptor) causing excessive production of cytokines which leads to a potentially fatal multisystem disease. (Both TSST and enterotoxin are examples of superantigens, described in detail in Chapter 10)

**Clinical features:** Patients present with fever, hypotension, mucosal (conjunctival) hyperemia, vomiting, diarrhea, confusion, myalgia, abdominal pain and erythematous rashes which desquamate later. Subsequently, there is rapid involvement of the other organs such as liver, kidneys, lungs, gastrointestinal tract (GIT) and/or central nervous system (CNS)

- Anti-TSST antibodies usually appear in the convalescent stage, they are protective in nature. TSS is more severe if anti-TSST antibodies fail to appear
- **Diagnosis:** Detection of TSST can be done by latex agglutination test and enzyme immunoassay. PCR-based assays are available for detection of TSST genes 1 and 2. Other findings may include altered liver/kidney function tests and low platelet count.

### TREATMENT

<table>
<thead>
<tr>
<th>Toxic shock syndrome</th>
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</thead>
<tbody>
<tr>
<td>As toxin causes capillary leak; aggressive parenteral fluid replacement should be initiated at the earliest</td>
</tr>
<tr>
<td>Examine for and remove any colonized foreign body, e.g. vaginal tampon</td>
</tr>
<tr>
<td>Clindamycin is the preferred drug for TSS (as it reduces the toxin synthesis). It is given along with anti-staphylococcal penicillin (e.g. cloxacillin) for MSSA or vancomycin for MRSA</td>
</tr>
<tr>
<td>For clindamycin resistant cases: Linezolid can be used for toxin suppression. In such case, addition of vancomycin is not required.</td>
</tr>
</tbody>
</table>
**Extracellular Enzymes**

**Coagulase**

The unique feature of _S. aureus_ is that it secretes coagulase enzyme which brings about clotting or coagulation of plasma.

- Coagulase enzyme combines with a plasma protein called CRF (coagulase reacting factor), and together they activate prothrombin, which in turn, converts fibrinogen to fibrin.
- This is the basis of tube coagulase test. This has to be differentiated from slide coagulase test, which is mediated by clumping factor (Table 21.3).
- Coagulase can react with rabbit or human plasma; but does not clot with guinea pig plasma as it lacks CRF.
- **Subtypes:** Coagulase enzyme has 8 antigenic types (A–H). **Type-A** is the most common type; secreted by human strains of _S. aureus_.

**Other Enzymes**

- Heat stable thermonucleases and DNase (deoxyribonuclease) are the enzymes that are specific to _S. aureus_; not produced by any other staphylococcal species.
- Staphylokinase (fibrinolysin) breaks down fibrin clots and may facilitate the spread of infection.
- Hyaluronidase breaks down the connective tissue network.
- Lipases and phospholipases breakdown the lipids.

**Pathogenesis**

Pathogenesis of _S. aureus_ involves the following steps:

- **Colonization:** _S. aureus_ colonizes on various body surfaces, such as anterior nares, axilla and perineal skin.
- **Introduction into the tissue:** Organisms are introduced into the tissues as a result of minor abrasions or instrumentation. Then they adhere to the tissue surfaces; which is mediated by various adhesins, e.g. clumping factor.
- **Invasion:** _S. aureus_ can invade into the tissues by elaborating enzymes, such as serine proteases, hyaluronidas, thermonucleases and lipases.
- **Evasion of host defense mechanisms:** _S. aureus_ exhibits various immune evasion mechanisms, such as:
  - Anti-phagocytic activity mediated by microcapsule and protein A
  - Inhibition of leukocyte migration (by chemotaxis inhibitory protein of staphylococci)
  - Intracellular survival inside the endothelial cells (by formation of small colony variants).
- **Metastatic spread:** Finally, _S. aureus_ spreads to various distant sites by hematogenous spread.

**Clinical Manifestations**

_Staphylococcus aureus_ is a pluripotent pathogen, causing various diseases through both toxin-mediated and non-toxin-mediated mechanisms. It is responsible for both nosocomial and community-based infections that range from relatively minor skin and soft tissue infections to life-threatening systemic infections (Table 21.4).

**Epidemiology**

_Staphylococcus aureus_ is a part of normal human flora. About 25–50% of healthy population are carriers of _S. aureus_, colonizing the organism persistently or transiently.

- **Most common site(s)** of colonization are anterior nares followed by skin (abraded), vagina, axilla, perineum, and oropharynx. These colonization sites serve as a reservoir for future infections.
- The rate of colonization is higher among insulin-dependent diabetics, HIV-infected patients, patients undergoing hemodialysis, and individuals with skin damage.
- Overall, _S. aureus_ is a leading cause of nosocomial infections. In hospitals, the health care professionals are the potential carriers of _S. aureus_. Hospital strains are

---

**Table 21.3:** Differences between tube and slide coagulase tests

<table>
<thead>
<tr>
<th>Tube coagulase</th>
<th>Slide coagulase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Due to coagulase enzyme</td>
<td>Due to clumping factor</td>
</tr>
<tr>
<td>Requires CRF in plasma</td>
<td>Does not require CRF in plasma</td>
</tr>
<tr>
<td>Test performed on tube</td>
<td>Test performed on slide</td>
</tr>
<tr>
<td>Positive if clot is formed</td>
<td>Positive if clumps are formed</td>
</tr>
<tr>
<td>Coagulase enzyme has eight serotypes</td>
<td>Clumping factor has one serotype</td>
</tr>
<tr>
<td><em>S. lugdunensis</em> gives a negative result</td>
<td><em>S. lugdunensis</em> gives a positive result</td>
</tr>
<tr>
<td>Both tube and slide coagulase positive for <em>S. aureus</em>, <em>S. hyicus</em>, and <em>S. intermedius</em></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviation: CRF, coagulase reacting factor.
Table 21.4: Clinical spectrum of *Staphylococcus aureus* infections

<table>
<thead>
<tr>
<th>Skin and soft tissue infections</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. aureus</em> is one of the most common cause of various skin and soft tissue infections such as:</td>
</tr>
<tr>
<td>- <strong>Folliculitis</strong> (infection of hair follicles)</td>
</tr>
<tr>
<td>- <strong>Furuncle (boil)</strong>: Painful pustular lesion in moist regions due to infection of the hair follicle</td>
</tr>
<tr>
<td>- <strong>Carbuncle</strong>: Severe, painful lesion in the lower neck region, extending to the deeper subcutaneous tissue</td>
</tr>
<tr>
<td>- <strong>Mastitis and breast abscess</strong> (in nursing mothers)</td>
</tr>
<tr>
<td>- <strong>Impetigo</strong>: It mainly occurs in children, usually appears as red sores on the face, that bursts and develops into <em>honey-colored crusts</em></td>
</tr>
<tr>
<td>- <strong>Surgical site wound infections</strong> (most common cause)</td>
</tr>
<tr>
<td>- <strong>Cellulitis</strong> (inflammation of skin and subcutaneous tissue) (Fig. 21.1)</td>
</tr>
<tr>
<td>- <strong>Hidradenitis suppurativa</strong>: A recurrent follicular infection in areas rich in apocrine glands, such as the axilla</td>
</tr>
<tr>
<td>- <strong>Botryomycosis</strong>: It is mycetoma-like condition, characterized by subcutaneous swelling, sinuses, and discharge containing granules (Described in Chapter 52).</td>
</tr>
</tbody>
</table>

Predisposing factors to *S. aureus* cutaneous infections are—chronic skin conditions (e.g. eczema), skin damage (trauma, injections) or poor personal hygiene

<table>
<thead>
<tr>
<th>Musculoskeletal infections</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. aureus</em> is the most common cause of various conditions such as:</td>
</tr>
<tr>
<td>- <strong>Septic arthritis</strong> (most commonly involved joints are knees, shoulders, hips, and phalanges)</td>
</tr>
<tr>
<td>- <strong>Osteomyelitis</strong> (most commonly affected site in children is long bones and in adults is vertebrae)</td>
</tr>
<tr>
<td>- <strong>Pyomyositis</strong> (<em>skeletal muscle infection</em>): In tropics and HIV infected people</td>
</tr>
<tr>
<td>- <strong>Abscess</strong>: Psoas abscess and epidural abscess.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Respiratory tract infections</th>
</tr>
</thead>
<tbody>
<tr>
<td>- Ventilator-associated pneumonia in adults</td>
</tr>
<tr>
<td>- Septic pulmonary emboli</td>
</tr>
<tr>
<td>- Postviral pneumonia (e.g. influenza)</td>
</tr>
<tr>
<td>- Empyema and pneumothorax</td>
</tr>
<tr>
<td>- Pneumatocele (shaggy, thin-walled cavities in lungs) in neonates: <em>S. aureus</em> is the most common cause</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Bacteremia and its complications</th>
</tr>
</thead>
<tbody>
<tr>
<td>- Sepsis, septic shock</td>
</tr>
<tr>
<td>- Central line associated blood stream infection (CLABSI)</td>
</tr>
<tr>
<td>- Metastatic foci of infection involving kidney, joints, bone and lung</td>
</tr>
<tr>
<td>- Infective endocarditis:</td>
</tr>
<tr>
<td>➢ Native-valve endocarditis—<em>S. aureus</em> is the most common cause</td>
</tr>
<tr>
<td>➢ Prosthetic-valve endocarditis</td>
</tr>
<tr>
<td>➢ Intravenous drug use associated endocarditis—<em>S. aureus</em> is the most common cause</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>UTI (Urinary tract infection)</th>
</tr>
</thead>
<tbody>
<tr>
<td>- Staphylococcal UTI and pyelonephritis usually occur secondary to bacteremia</td>
</tr>
<tr>
<td>- Rarely UTI is seen following instrumentation and insertion of catheter or implants</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Toxin-mediated illnesses (Described earlier)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. aureus</em> causes the following toxin mediated diseases (as described earlier):</td>
</tr>
<tr>
<td>- Toxic shock syndrome</td>
</tr>
<tr>
<td>- Food poisoning</td>
</tr>
<tr>
<td>- Staphylococcal scalded-skin syndrome</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Infections associated with CA-MRSA (Community associated methicillin-resistant <em>Staphylococcus aureus</em>)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skin and soft tissues are the most common sites for colonization of CA-MRSA strains; about 5–10% of strains are invasive and can cause various invasive infections, such as:</td>
</tr>
<tr>
<td>- Necrotizing pneumonia</td>
</tr>
<tr>
<td>- Sepsis with Waterhouse-Friderichsen syndrome or purpura fulminans (<em>S. aureus</em> is rare cause; most commonly caused by meningococcus).</td>
</tr>
<tr>
<td>- Necrotizing fasciitis (<em>S. aureus</em> is a rare cause, <em>Streptococcus pyogenes</em> is the most common cause)</td>
</tr>
</tbody>
</table>
often multidrug resistant, spread to patients either from hospital staff/other patients/environment or also from patient’s own endogenous flora.

**LABORATORY DIAGNOSIS**

**Staphylococcus aureus**

- **Direct smear microscopy**: Gram-positive cocci in clusters and pus cells.
- **Culture**
  - Nutrient agar—golden yellow pigmented colonies
  - Blood agar—colonies with narrow zone of β-hemolysis
  - Selective media—such as mannitol salt agar, salt milk agar and Ludlam’s medium.
- **Culture smear microscopy**: Gram-positive cocci in clusters.
- **Biochemical identification**: Catalase test-positive.

Tests differentiating *S. aureus* (gives a positive result) from CoNS (gives a negative result):

- Coagulase test (slide and tube)—positive
- Heat stable thermo nuclease test—positive
- DNase test—positive
- Mannitol sugar is fermented
- Black-colored colonies on potassium tellurite agar
- Gelatin liquefaction—positive
- Protein A detection.
- **Typing methods**
  - Phenotypic methods such as bacteriophage typing and antibiogram typing
  - Genotypic methods such as PCR-RFLP.
- **Antimicrobial susceptibility testing**.

**Laboratory Diagnosis**

**Sample Collection**

It depends on the nature of the lesion (Table 21.5).

**Direct Smear Microscopy**

Gram staining of pus or wound swab reveals pus cells with gram-positive cocci in clusters (Fig. 21.2A). However, direct microscopy is of no value when *S. aureus* is a part of normal flora in the sample (e.g. sputum or feces).

**Culture**

Specimens are inoculated onto various media and incubated overnight at 37°C aerobically. The colony morphology is observed as follows:

- **Nutrient agar**: Colonies are 1–3 mm in size, circular, smooth, convex, opaque and easily emulsifiable. Most strains produce golden yellow non-diffusible pigments (made up of β carotene) (Fig. 21.3A)
- **Nutrient agar slope**: It produces golden yellow colonies of confluent growth, looks like oil paint appearance
- **Blood agar**: Colonies are similar to that on nutrient agar, in addition surrounded by a narrow zone of β hemolysis (best observed in sheep blood agar) (Fig. 21.3B)
- **MacConkey agar**: Small pink colonies are produced due to lactose fermentation
- **Liquid medium (e.g. peptone water)**: It produces uniform turbidity
- **Selective media**: They are useful when staphylococci are expected to be scanty or outnumbered by other bacteria in the sample (e.g. swabs from carriers, feces). Salt is added to the media, as it is inhibitory to other bacteria but not to staphylococci. Examples include:
  - Mannitol salt agar contains nutrient agar with 7.5% NaCl and phenol red as an indicator. All staphylococci can grow at 7.5% salt; however, *S. aureus* produces yellow-colored colonies due to mannitol fermentation (Fig. 21.3C)
  - Salt milk agar contains nutrient agar, 6.5% NaCl and 10% skimmed milk
  - Ludlam’s medium contains lithium chloride and tellurite.

**Culture Smear Microscopy**

Gram staining from the colonies shows Gram-positive cocci (1 µm), arranged in clusters (Fig. 21.2B). Hanging drop reveals non-motile cocci.

![Image](image-url)
CHAPTER 21  Staphylococcus

Biochemical Tests for Identification

Catalase Test
All members of Micrococcaceae (staphylococci and micrococci) are catalase positive, which differentiates them from Streptococcaceae (catalase negative).

Hugh and Leifson Oxidative Fermentative Test
This test differentiates staphylococci (shows fermentative pattern) from micrococci (shows oxidative pattern).

Tests to Differentiate S. aureus from CoNS
S. aureus can be differentiated from CoNS (coagulase-negative staphylococci) by various tests (as described in the laboratory diagnosis box), of which the coagulase test is most important (see Table 21.3).

Coagulase Test
It is the most commonly used biochemical reaction for identification of S. aureus.

Tube Coagulase Test
It detects free coagulase secreted by S. aureus.
- **Procedure:** Colony of S. aureus is emulsified in 1 mL of diluted plasma (1:6) in a test tube and incubated at 37°C, preferably in a water bath for up to 4 hours
- **Positive test** is indicated by formation of a clot that does not flow when the test tube is tilted (Fig. 21.4A). Any amount of clot formation is considered as positive
- The **negative tubes** (no clot formation) should be incubated overnight and re-examined as some strains may produce a delayed clot (Fig. 21.4B)
- **False-positive:** Citrated plasma should not be used as some bacteria (e.g. Pseudomonas) may utilize citrate and give a false positive result. Heparin or EDTA are the preferred anticoagulants.

Slide Coagulase Test
It detects clumping factor (i.e. bound coagulase).
- **Procedure:** A colony of S. aureus is emulsified with a drop of normal saline on a clean slide to form a milky white suspension. Then a loopful of undiluted plasma is added and mixed properly
- **Positive result** is indicated by formation of coarse clumps (Fig. 21.4C)
- **Results** should be confirmed by the tube coagulase test as ≥15% of S. aureus strains (including some MRSA) give false-negative results. At the same time, few CoNS, such as S. lugdunensis give a positive result.

DNase Test
On DNA agar, a clear halo is produced surrounding the colonies of S. aureus, due to its ability to digest DNA.

Phosphatase Test
This test is positive for S. aureus, S. epidermidis and S. xylosus. Organism is inoculated on phenolphthalein

Figs 21.3A to C: Colonies of S. aureus A. Nutrient agar—shows golden-yellow pigmented colonies; B. Blood agar—arrow shows narrow zone of beta hemolysis surrounding the colonies; C. Mannitol salt agar shows yellow-colored colonies of S. aureus due to fermentation of mannitol
Source: Department of Microbiology, Pondicherry Institute of Medical Sciences, Puducherry (with permission).

Figs 21.4A to C: Coagulase test: A. Tube coagulase test (positive); B. Tube coagulase test (negative); C. Slide showing coagulase test
Source: Department of Microbiology, Pondicherry Institute of Medical Sciences, Puducherry (with permission).
diphosphate containing media and later the colonies grown are exposed to ammonia vapor (see below).

\[ S. aureus \rightarrow \text{splits phenolphthalein diphosphate in the media} \rightarrow \text{releases free phenolphthalein} \rightarrow \text{reacts with ammonia vapors} \rightarrow \text{colonies turn pink.} \]

Typing of *S. aureus*

Typing of *S. aureus* to subspecies level is done for epidemiological purpose to trace the source of infection. It is especially useful in outbreaks such as food poisoning affecting a larger community. Typing methods include both:

- Phenotypic methods such as bacteriophage typing and antibiogram typing
- Genotypic methods such as PCR-RFLP (restricted fragment length polymorphism), ribotyping, PFGE (pulse field gel electrophoresis) and sequence based typing.

Bacteriophage Typing

Strains of *S. aureus* can be further differentiated into subspecies level based on their susceptibility to bacteriophages (pattern method of phage typing).

- Phage type 80/81 is most commonly associated with outbreaks in hospitals. It is known as epidemic strain of *S. aureus*
- With the advent of molecular typing methods, phage typing has become obsolete nowadays
- Refer author’s first edition for detail procedure.

Antimicrobial Susceptibility Test

As *S. aureus* develops resistance to antibiotics readily, drugs should be prescribed according to the antimicrobial susceptibility test done on Mueller Hinton agar.

Drug Resistance in *S. aureus* 
(Resistance to β-lactam antibiotics)

*Staphylococcus aureus* shows resistance to β-lactam antibiotics in various way.

Production of β Lactamase Enzyme

β-lactamase or penicillinase enzymes cleave the β lactam rings, and there by organisms producing these enzymes develop resistance to β lactam antibiotics.

- This resistance is plasmid coded, can be transferred between *S. aureus* strains by transduction
- It is produced by >90% of strains of *S. aureus*
- This resistance can be overcome by addition of β-lactamase inhibitors such as clavulanic acid or sulbactam.

By Alteration of Penicillin-Binding Protein (PBP)

It is shown by MRSA strains of *S. aureus*.

TREATMENT

<table>
<thead>
<tr>
<th>Staphylococcus aureus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Since <em>S. aureus</em> rapidly develops drug resistance, antibiotics should be cautiously chosen.</td>
</tr>
</tbody>
</table>

Parenteral therapy for serious infections

<table>
<thead>
<tr>
<th>Sensitive to pencillin</th>
<th>DOC: Penicillin G</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methicillin sensitive</td>
<td>DOC: Anti-staphylococcal penicillins such as nafcillin and cloxacillin</td>
</tr>
<tr>
<td><em>S. aureus</em> (MSSA)</td>
<td>Note: Vancomycin is inferior in terms of efficacy against MSSA, when compared to anti-staphylococcal penicillins; hence should not be prescribed for MSSA</td>
</tr>
<tr>
<td>Methicillin resistant</td>
<td>DOC: Vancomycin (15–20 mg/kg bd)</td>
</tr>
<tr>
<td><em>S. aureus</em> (MRSA)</td>
<td>Alternate drugs:</td>
</tr>
<tr>
<td></td>
<td>- Teicoplanin</td>
</tr>
<tr>
<td></td>
<td>- Daptomycin (for endocarditis and complicated skin infections, not used for pneumonia)</td>
</tr>
<tr>
<td></td>
<td>- Linezolid and telavancin</td>
</tr>
<tr>
<td></td>
<td>- Quinupristin/dalfopristin</td>
</tr>
<tr>
<td></td>
<td>Note: All β lactam drugs should be avoided except newer cephalosporins (e.g. ceftobiprole)</td>
</tr>
</tbody>
</table>

Empirical therapy (if MRSA status not yet known):

Vancomycin with/without an aminoglycoside (vancomycin is indicated only if MRSA risk is high or condition is serious, e.g. cardiac implant).

Oral therapy for skin and soft tissue infections

| Sensitive to methicillin | - Dicloxacillin |
|                         | - Cephalexin/cefazolin |
| Resistant to methicillin (MRSA) | - Clindamycin |
| Alternate drugs:       | Cotrimoxazole, doxycycline, Linezolid |

Abbreviations: DOC, drug of choice; bd, twice a day.

Methicillin-resistant *Staphylococcus aureus* (MRSA)

Methicillin resistance in *S. aureus* is mediated by a chromosomally coded gene called *mec A gene*, which alters penicillin-binding protein (PBP) present on *S. aureus* cell membrane to PBP-2a.

- PBP is an essential protein needed for cell wall synthesis of bacteria. β lactam drugs bind and inhibit this protein, there by inhibit the cell wall synthesis
- The altered PBP2a of MRSA strains has less affinity for β lactam antibiotics; hence, MRSA strains are resistant to all β lactam antibiotics
- Recently, *mec C* gene (coding for PBP-2c) has also been found to be associated with MRSA
- Borderline oxacillin resistant *S. aureus* (BORSA) strains: Occasionally a non-*mec A* gene mediated low level resistance to oxacillin is observed in some strains of
Emergence of resistance to vancomycin. It may be of low
Erroneous and overuse of vancomycin has lead to the
Resistance to Vancomycin (VRSA and VISA)

Detection of MRSA

MRSA are either community or hospital associated (Table 21.6).

Types of MRSA

MRSA are either community or hospital associated (Table 21.6).

Detection of MRSA

Antimicrobial susceptibility test: Disk diffusion test can be done by using cefoxitin disc
Oxacillin screening agar: Adding oxacillin 6 μg/mL and NaCl (2–4%) to the Mueller Hinton agar and incubated at 30°C for full 24 hours
PCR detecting mecA gene
Latex agglutination test detecting PBP-2a.

<table>
<thead>
<tr>
<th>Table 21.6: Types of MRSA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Community-associated MRSA (CA-MRSA)</td>
</tr>
<tr>
<td>These strains express mecA gene subtype IV, V, VI</td>
</tr>
<tr>
<td>They are usually more virulent and express several toxins such as Panton Valentine (PV) toxin</td>
</tr>
<tr>
<td>They cause invasive skin and soft tissue infections such as necrotizing fasciitis (see Table 21.4)</td>
</tr>
</tbody>
</table>

Note: CA-MRSA and HA-MRSA terminologies are losing their relevance; as many CA-MRSA strains have been isolated in hospitals and vice versa.

S. aureus. This is believed to be due to hyperproduction of β-lactamase

Prevalence: MRSA infection rate has been increasing over last few decades, though it varies from place to place. MRSA rates are higher (>50%) in America, some Asian and European countries and Malta. Countries with lowest MRSA rates are Netherlands and Scandinavia (<1%). In India, the MRSA rate is around 30–40%; though varies between years and place.

Types of MRSA

MRSA are either community or hospital associated (Table 21.6).

Detection of MRSA

Antimicrobial susceptibility test: Disk diffusion test can be done by using cefoxitin disc
Oxacillin screening agar: Adding oxacillin 6 μg/mL and NaCl (2–4%) to the Mueller Hinton agar and incubated at 30°C for full 24 hours
PCR detecting mecA gene
Latex agglutination test detecting PBP-2a.

<p>| MRSA |</p>
<table>
<thead>
<tr>
<th>TREATMENT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vancomycin is the drug of choice for MRSA</td>
</tr>
<tr>
<td>Alternate drugs include—teicoplanin, linezolid, daptomycin, telavancin and quinupristin/dalfopristin</td>
</tr>
<tr>
<td>However, even drugs such as tetracycline, erythromycin or cotrimoxazole may also be effective in non life-threatening infections</td>
</tr>
<tr>
<td>Antimicrobial susceptibility testing is necessary before an alternative drug is used</td>
</tr>
<tr>
<td>For nasal carriers of MRSA, mupirocin (2%) ointment is used. All β-lactam drugs should be avoided. However, 5th generation cephalosporins, such as cefotiprole, ceftriaxone and ceftolozane have shown some activity against MRSA.</td>
</tr>
</tbody>
</table>

Resistance to Vancomycin (VRSA and VISA)

Erroneous and overuse of vancomycin has lead to the emergence of resistance to vancomycin. It may be of low grade resistance, known as VISA (vancomycin intermediate S. aureus) or high-grade resistance, known as VRSA (vancomycin-resistant S. aureus).

The MIC (minimum inhibitory concentration) of vancomycin to VISA and VRSA isolates are 4–8 and >8 μg/mL respectively

MIC creep: It is observed that the vancomycin MIC for susceptible strains of S. aureus has been gradually increasing over time (known as MIC creep); which indicates that the frequency of VISA and VRSA is likely to increase in future. Current guidelines recommend consideration of alternative drugs if vancomycin MIC is >1 μg/mL as treatment failure has been frequently observed beyond this MIC level

VRSA is very rare. In India, it is reported from few places such as Hyderabad, Kolkata and Lucknow. However, VISA is more frequently reported than VRSA

Mechanisms: VRSA is mediated by van A gene; whereas VISA is due to increase in cell wall thickness of S. aureus. The van A gene is believed to be acquired from a vancomycin-resistant strain of Enterococcus faecalis by horizontal conjugal transfer

Fitness cost: Acquisition of a van gene is often associated with compensatory mutations in the genes responsible for survival which results in a reduced fitness of S. aureus. In contrast, ‘fitness cost phenomena’ is not commonly observed in MRSA. This explains why VRSA is seen very rarely (<0.1%), whereas MRSA prevalence is so common (30–40%)

Treatment of VRSA should be based on antimicrobial susceptibility report. Linezolid, telavancin, daptomycin and quinupristin/dalfopristin are the effective drugs.

Control Measures

Prevention of spread of S. aureus infections in hospitals involves:

Screening of MRSA carriers among hospital staff should be done when there is an outbreak. Mannitol oxacillin agar is the preferred media for this purpose

Treatment of carriers is done by use of topical mupirocin (for nasal carriers) and chlorhexidine (for skin carriers)

Stoppage of antibiotic misuse in hospitals

Ensure proper infection control measures such as hand hygiene (most efficient way to prevent hospital spread), isolation of the patients and all other measures of contact precautions (described in detail in Chapter 53).

COAGULASE-NEGATIVE STAPHYLOCOCCII

Most of the coagulase-negative staphylococci (CoNS) are harmless commensals and less virulent than S. aureus; however, recently their role as pathogen is increasingly been reported.
**Staphylococcus epidermidis**

It is the most common CoNS (75–80%), isolated from clinical samples. It is present as normal flora on the skin, oropharynx and vagina; however, its pathogenic role is greatly enhanced in presence of prosthetic-devices.

- **Pathogenesis:** *S. epidermidis* involves a two-step process:
  1. **Initial adhesion to the prosthetic device:** The surface adhesins of the organism bind to host serum or tissue constituents, such as fibrinogen or fibronectin, coated on the implanted prosthetic surfaces.
  2. **Colonization:** *S. epidermidis* can produce the extracellular polysaccharide material (glycocalyx or slime) that facilitates formation of a protective biofilm on the device surfaces. Biofilm appears to act as a barrier, protecting bacteria from host defense mechanisms as well as from antibiotics.

- **Manifestation:** *S. epidermidis* is the most common cause of prosthetic-device related infections, such as endocarditis with insertion of valvular prosthesis and ventricular shunt infections. It is also a common cause of stitch abscess.

- It is coagulase negative, but positive for phosphatase test.

**Staphylococcus saprophyticus**

It causes urinary tract infection (UTI) in sexually active young women. This is due to expression of a 160 kDa hemagglutinin/adhesin protein that can adhere to uroepithelial cells. It can be differentiated from other staphylococci in being resistant to novobiocin disk (5 µg).

**Staphylococcus lugdunensis and Staphylococcus schleiferi**

Recently, these organisms have been associated with more serious infections such as native-valve endocarditis and osteomyelitis. Their enhanced pathogenesis may be due to expression of virulence factors such as clumping factor and lipase which are usually absent in other CoNS.

**Laboratory Diagnosis of CoNS**

Various species of CoNS can be differentiated from each other and also from *S. aureus* by various biochemical tests (Table 21.7). Treatment is same as that of *Staphylococcus aureus*.

---

**Table 21.7: Tests for identification of common *Staphylococcus* species**

<table>
<thead>
<tr>
<th>Properties</th>
<th><em>S. aureus</em></th>
<th><em>S. epidermidis</em></th>
<th><em>S. saprophyticus</em></th>
<th><em>S. lugdunensis</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Coagulase (tube)</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Clumping factor</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Heat stable thermonuclease</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Phosphatase</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Novobiocin</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>S</td>
</tr>
<tr>
<td>Urease</td>
<td>V</td>
<td>+</td>
<td>+</td>
<td>V</td>
</tr>
<tr>
<td>PYR</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Ornithine decarboxylation</td>
<td>–</td>
<td>–</td>
<td>–</td>
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Abbreviation: PYR, pyrrolidonyl-beta-naphthylamide.

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**I. Essay:**

1. A 55-year-old male was admitted to the hospital with complaints of severe pain in the lateral aspect of his left calf and small amount of pus discharge from the ingrown hair. On physical examination, the local area was found to be red, warm and tender. Pus was aspirated and was subjected to Gram stain (showed gram-positive cocci in clusters), culture on blood agar (showed golden yellow pigmented beta hemolytic colonies).

   a. What is the clinical diagnosis and its causative organism?
   b. List the infections caused by this organism.
   c. List the virulence factors of this organism.
   d. Briefly discuss the laboratory diagnosis.

**II. Write short notes on:**

1. Toxic shock syndrome
2. Staphylococcal food poisoning
3. MRSA (Methicillin-resistant *Staphylococcus aureus*)

**III. Multiple Choice Questions (MCQs):**

1. Scalded skin syndrome is mediated by:
   a. Hemolysin  
   b. Coagulase  
   c. Enterotoxin  
   d. Epidermolytic toxin

2. *Staphylococcus aureus* causes vomiting in 6–8 hours. The mechanism of action is by:
   a. Stimulation of cAMP  
   b. Vagal stimulation  
   c. Stimulation of cGMP  
   d. Acts through ganglioside GM receptor
3. A patient has prosthetic valve replacement and he develops endocarditis 8 months later. Organism responsible is?
   a. *Staphylococcus aureus*
b. Viridans streptococci
c. *Staphylococcus epidermidis*
d. HACEK
4. Which hemolysin of *S. aureus* shows hot-cold phenomenon?
   a. α 
   b. β
   c. γ 
   d. δ
5. Which hemolysin of *S. aureus* is inactivated at 70°C but again reactivated paradoxically at 100°C?
   a. α 
   b. β
   c. γ 
   d. δ
6. Synergohymenotropic toxins includes:
   a. α-hemolysin and panton valentine toxin
   b. β-hemolysin and panton valentine toxin
   c. γ-hemolysin and panton valentine toxin
   d. α-hemolysin and γ-hemolysin
7. Coagglutination reaction is mediated by which component of *S. aureus*?
   a. Mec A 
   b. Protein A
   c. Coagulase 
   d. Clumping factor
8. *Staphylococcus aureus* enterotoxin, all are true, except:
   a. Preformed toxin 
   b. Incubation period is short (1–6 hours)
   c. The toxin stimulates the vagus nerve and the vomiting center of the brain
   d. Treatment is mainly by early institution of antibiotics
9. Tube coagulase differs from slide coagulase by all, except:
   a. Requires coagulase reacting factor in plasma 
   b. *S. lugdunensis* gives a positive result
   c. Positive result is indicated by clot formation
   d. Coagulase enzyme has eight subtypes
10. CA-MRSA strains are increasingly associated with all of the following, except:
    a. Necrotizing pneumonia 
    b. Waterhouse-Friderichsen syndrome
    c. Necrotizing fasciitis 
    d. Toxic shock syndrome
11. *S. aureus* is differentiated from CoNS by all, except:
    a. Coagulase test 
    b. DNase test
    c. Catalase test 
    d. Protein A detection
12. Which sugar fermentation test differentiates *S. aureus* from CoNS?
    a. Glucose 
    b. Sucrose
    c. Lactose 
    d. Mannitol
13. All of the above can be given for the treatment of MRSA, except:
    a. Meropenem 
    b. Vancomycin
    c. Cotrimoxazole 
    d. Linezolid
14. All the following beta lactam drugs can be given for the treatment of MRSA, except:
    a. Cefaroline 
    b. Cefotibiprole
    c. Piperacillin-tazobactum 
    d. Cefotolozane
15. Which is the least preferred antimicrobial for the treatment of methicillin-sensitive *S. aureus* (MSSA)?
    a. Dicloxacillin 
    b. Cephalexin
    c. Cefazolin 
    d. Vancomycin
16. About MRSA all are true, except:
    a. In India, the MRSA prevalence in hospital is around 30–40%
    b. MRSA rates are higher in India than in America
    c. Mediated by Mec A gene
    d. Cefoxitin disk is superior to oxacillin for detection
17. About VRSA all are true, except:
    a. VRSA is mediated due to Van gene 
    b. VISA is due to increased cell wall thickening 
    c. VRSA is more common than VISA
    d. Fitness cost phenomena is seen in VRSA
18. Borderline oxacillin resistant *S. aureus* (BORSA) strains, the mechanism of resistance is due to:
    a. Mec A gene mediated 
    b. Alteration of penicillin binding protein
    c. Hyperproduction of β lactamase
    d. Van gene mediated
19. Community-associated MRSA (CA-MRSA) differs from hospital-associated MRSA by all, except:
    a. These strains express mec A gene subtype IV, V, VI
    b. Express more Panton Valentine (PV) toxin
    c. They cause more invasive skin and soft tissue infection
    d. Multidrug resistant
20. *Staphylococcus epidermidis*, all are true, except:
    a. Accounts for 75% of CoNS 
    b. Phosphatase negative 
    c. Produces biofilm
    d. Causes stitch abscesses
21. Positive tube coagulase test is a property of all the following species of *Staphylococcus*, except:
    a. *S. aureus* 
    b. *S. hyicus* 
    c. *S. intermedius* 
    d. *S. lugdunensis*